

Research Article

COMPARATIVE HAEMOSTATIC EFFICACY OF SUCCULENT LEAF EXTRACTS AND LATEX OF SOME WOUND HEALING PLANTS ON FRESH WOUND OF RABBIT

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ABSTRACT: Ethnomedicinal report of haemostatic activity of six medicinal plants was validated by a study of the effect of succulent leaf extract of plant parts on the punch wound of rabbit for the first time. It was found that the succulent leaf extracts of *Artemisia nilagirica* (Clarke), *Barleria lupulina* Lindl., *Blumea lacera* Dc., *Croton bonplandianum* Baill., *Glinus lotoides* Lin. and *Mikania scandens* (L) Willd. can induce haemostasis in fresh wounds as compared to automatic haemostasis (120.00 \pm 2.91 seconds). The fresh leaf extract of *Mikania scandens* took 25.00 \pm 1.87 seconds for haemostatic activity. *Artemisia nilagirica* (35.00 \pm 1.50 seconds), *Barleria lupulina* (30.00 \pm 2.34 seconds), *Blumea lacera* (38.00 \pm 1.87 seconds), *Glinus lotoides* (35.00 \pm 2.29 seconds) are having better action than *Croton bonplandianum* (leaf extract), which took 40.00 \pm 2.69 seconds time for haemostasis. The latex collected from the wounded small branches of living *Croton bonplandianum* plant is having highest efficacy in causing haemostasis (10.00 \pm 1.22 seconds), better than the positive control of Tincture Ferric per Chloride (13.00 \pm 2.54 seconds). The dermal toxicity study reveals that the application of the fresh plant extract on the skin of rat failed to produce any detrimental effect. The plant extracts collected from succulent plant leaves and particularly the latex collected from the living *Croton bonplandianum* Baill. plant can be used as haemostatic agents.

Key words: Haemostasis, Ethnomedicine, Succulent leaf extracts, *Croton bonplandianum* Baill., Latex.

INTRODUCTION

Natural products have been the source of

most of the active ingredients of medicines. This is widely accepted to be true when applied to

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drug discovery in 'olden times'. More than 80% of drug substances were natural products or inspired by a natural compound (Sneder 1996). Comparisons of the information presented on sources of new drugs from 1981 to 2007 indicate that almost half of the drugs approved since 1994 are based on natural products (Newman and Cragg 2007; Butler 2008).

For drug discovery from medicinal plants, studies are performed to validate the reported medicinal property of plants. Generally the plant parts were collected, dried and preserved. Then methanolic, ethanolic, acetone, aqueous etc. extracts of the preserved plant parts were made and stored at different manner. Then these were tested for their reported medicinal use *in vitro* or among *in vivo* animal models, either in that form or in semi-purified or purified form after identification of active principles (Pattanayak *et al.*, 2013). But the ethnic and other rural people traditionally use the plants in its crude, fresh form in most of the time. It was argued that the concept of contemporary research on dry plant parts have the limitation of loss of many aromatic and other phytochemicals present in the living plant, which may have very important role when used together (Pattanayak *et al.*, 2013).

A total of six medicinal plants used for wound healing were selected on the basis of published literature to evaluate the haemostatic activity of the plant extracts collected from the succulent plant parts taken from the living plants.

MATERIALS AND METHODS

The plants

1. *Artemisia nilagirica* (C. B. Clarke) Pamp. (F. Compositae/Asteraceae).

Name: Bengali- Nakdana, Damanaka. Hindi- Nagdona, English-Indian worm wood.

Collected from : Goas, Murshidabad.

Uses: The plant possesses antiseptic property (Chopra *et al.*, 1982), fresh leaf extract is used to check nasal bleeding of children and also used in domestic animals (Pattanayak *et al.*, 2012).

2. *Barleria lupulina* Lindl. (F: Acanthaceae)

Name: Bengali-Vishalyakarani. English-Hophead, Philippine violet.

Collected from : Saratpalli, Belda, Paschim Medinipur.

Uses: The juice of leaves of this plant is used to stop instant bleeding. It is also used to cure all types of wound and ulcers, both fresh and old (Pattanayak *et al.*, 2012) and is having antibacterial effect (Pattanayak *et al.*, 2014). The plant is externally used as an anti-inflammatory against insect bites, snake bites, herpes simplex, herpes zoster and varicella zoster virus lesions and it also has a diuretic effect and anti-amoebic activities (Kanchanapoom *et al.*, 2001, Lans *et al.*, 2001, Sawangjaroen *et al.*, 2006). The methanolic extract of this plant is having anti-ulcer activity (Suba *et al.*, 2004).

3. *Blumea lacera* (Burm. f.) DC. (F: Compositae/Asteraceae).

Name: Bengali-Kuksima/Kukursunga, Hindi-Kukkurbanda. English – Blume.

Collected from : Ramchandrapur, Purba Medinipur.

Uses: Leaf used in cuts and wounds (Gupta *et al.*, 2010). Plant is used to cure wounds of animals. The juice of the whole plant (including the root) is applied regularly on large-sized wound after slight heating 2-3 times daily to cure wounds and to protect the animal from development of problems like tetanus and gangrene (Pattanayak *et al.*, 2012).

4. *Croton bonplandianum* Baill. (F: Euphorbiaceae).

Name: Bengali-Bana-tulasi. Hindi – Jungle tulsi. English – Three-leaved caper.

Collected from : Madhyahinghli, Mahisadal.

Uses: It is a healing plant (Parrotta 2001).

The leaves of the plant is used for controlling high blood pressure, skin diseases and cut and wounds (Jeeshna *et al.*, 2010). The oozing out fresh latex of the plant is used to check bleeding from all types of fresh wound (Pattanayak *et al.*, 2012).

5. *Glinus oppositifolius* (L.) Aug. DC.(F: Molluginaceae)

Name: Bengali-Gimesag/Duserasag, Hindi – Jima. English – Indian Chickweed, Bitter leaf.

Collected from : Projabharh, Purba Medinipur .Uses : Poultice of leaves is applied on wound and inflammation (Qureshi *et al.*, 2010).The leaf extract is applied on wounds to check bleeding and to cure the wound quickly. The applied quantity is according to the requirement (Pattanayak *et al.*, 2012).

6. *Mikania scandens* (L.) Willd. (F: Asteraceae)

Name: Bengali-Banchhalata/Taralata, English-Climbing hempweed.

Collected from : Ramchandrapur, Purba Medinipur.

Uses : The leaf paste and leaf juice is used in wound and bruises (Nawaz *et al.*, 2009). Juice of fresh leaves is used as haemostatic agent on fresh wounds in West Bengal, India (Pattanayak *et al.*, 2012).Aqueous leaf extracts of this plant have been used in folk medicine to treat stomach ulcers (Dey *et al.*, 2011). The extract of succulent leaves of this plant is having antimicrobial and wound healing property in punch wound of rabbit (Pattanayak *et al.*, 2015).

Collection of plant material

After identification of the plants by taxonomist, the plant parts were collected during the month of September- October, 2013.

Preparation of fresh extract of the plant parts

As the rural people use plant parts as a paste on the affected body parts most of the time for wound healing, almost the same procedure was followed during the extraction of fresh plant extracts from the plant parts. Only the rough portion of the plant parts was excluded. Hundred grams of the clean, washed leaves of six plants [*Artemisia nilagirica* (Clarke), *Barleria lupulina* Lindl, *Blumea lacera* Dc., *Croton bonplandianum* Baill., *Glinus oppositifolius* (L.) Aug. DC., *Mikania scandens* (L) Willd.] were taken and rubbed slightly individually with wet cotton to remove adhered dust particles. Then those were air dried for a few minutes and paste of the plant material (leaves) were made by pestle and mortar and afterwards the material were filtered by three layers of clean, white cotton cloth, centrifuged at 1000 rpm for 10 minutes and stored in airtight bottles as small aliquots at 4°C for two hours and then at - 20°C for further use.

Preparation of latex of *Croton bonplandianum* Baill.

The oozing out latex of the plant was directly collected after breaking the small branches of the plant. Then it was stored at – 20°C as small aliquots.

Preparation of animal for the study

The present study was performed with permission of Institutional Ethics Committee for use of animals in the experiment.

Animals: New Zealand variety Rabbit of both sexes housed in laboratory conditions.

A total of 12 animals of both sexes within the age group of 12-18 months were taken for the study. Three wounds were created on each the side of backbone mark. A total of $12 \times 6 = 72$ wounds were created for the study. Eight

wounds were used for each of the nine parameters (six plant extracts, one latex, one positive control of Tincture Ferric per Chloride and one parameter for automatic haemostasis). Each plant extract as well as the controls were tested in four animals. One wound at each side of the backbone of four rabbits were treated with one plant extract. So, rabbit no. 1 to 4 were used for plant extract of serial no. 1 to 3. Likewise, next four rabbits were used plant extract of serial no. 4 to 6. Another four rabbits were used for plant extract no. 7 and two controls.

Procedure of haemostatic efficacy study

The rabbits were anesthetized by intramuscular injection of Xylazine (10 mg/kg body weight) and Ketamin (40 mg/ kg body weight). The hair of the animals was removed carefully with blade.

Then Punch wounds were prepared in anaesthetized clean animals by an 8 mm. diameter skin biopsy punch [Acu Punch^R of Acuderm Inc (U.S.A.)] on either side of the backbone mark.

After creation of each wound, three drops of fresh extract of leaves of the plants under study, the latex of *Croton bonplandianum* Baill. and Tincture Ferric per Chloride, the positive control was added on it as per the protocol. Some wounds were not added with any medicine to observe the time required for automatic haemostasis. The wounds were observed under magnifying glass (75 mm) to note the time interval of stoppage of bleeding and increase of total volume of liquid on the wound surface.

Dermal toxicity study

The toxicity study of the fresh plant extract was performed as per the standard protocol (OECD guideline, 1987).

Adult male Rats, weighing between 150 - 200 grams were taken for the study. Five rats were

taken in each group of eight groups used for seven types of fresh extract and one control group kept without medicine. They were kept 7 days in the animal house for acclimatization. The temperature of the animal house was kept between 22°C to 25°C. The animals were kept in light for 12 hours daily. Common rat diet of the animal house and unlimited supply of water was provided for the animals.

Test Procedure

For restraining of the animals, injection of Ketamine @ 50 mg/Kg body weight and Xylazine @ 8 mg/kg body weight intramuscularly given before starting experiment. The fur of the rats was removed by VEET^R [Rackitt Benckiser (India) Limited] ointment application before the experiment. The body parts were washed by distilled water thoroughly after removal of hairs. More than 10% of the body part of the test animals was made hair free by that process.

For testing of possible toxic effect of the plant extracts, the fresh plant extract was used as such, undiluted. The animals were treated with fresh plant extracts on the skin surface and these were kept in close contact with the skin with a porous gauze dressing and non irritating tape for 24 hours. The fresh extracts were changed daily for 14 days. Regular observation of the skin of the rats under test was performed with the help of a magnifying glass to observe any type of abnormality of the skin structure like appearance of any reddish spot, pimples, edematous swelling etc.

RESULT AND DISCUSSION

Haemostasis is one of the factors related with wound healing. The haemostatic efficacy study of the plant extracts collected from living plant on rabbit is reported for the first time here. So, no data of previous reporting is available for

comparison. It was found that among the fresh extract and latex tested, the latex collected from the plant *Croton bonplandianum* Baill. is having highest activity in checking of blood loss (10 seconds) from the fresh wound followed by the positive control of Tincture Ferric Per Chloride (13 seconds). The fresh leave extract of *Mikania scandens* (L) Willd. took some more time (25 seconds) for haemostatic activity. Extracts collected from other four plants showed better activity of haemostasis as compared to automatic haemostasis. *Artemisia nilagirica* (Clarke) (35 seconds), *Barleria lupulina* Lindl. (30 seconds), *Blumea lacera* Dc. (38 seconds), *Glinus lotoides* Lin. (35 seconds) are having better activity than the effect of *Croton bonplandianum* Baill. (leaf extract), which took 40 second time for haemostasis (Table 1).

The results of dermal toxicity study reveal that the application of the fresh plant extract on the skin of rat failed to produce any detrimental effect, either externally or internally.

Haemostasis is a process which causes bleeding to stop. It is the first stage of the process of healing of wound. This involves changing of the form of blood from liquid to gel. Intact blood vessels are central to moderating blood's tendency for clotting. The cells of intact vessels prevent blood clotting with a heparin-like molecule and thrombomodulin and prevent platelet aggregation with nitric oxide and prostacyclin. When endothelial injury occurs, the endothelial cells stop secretion of coagulation and aggregation inhibitors and instead secrete von willebrand factor which initiate the maintenance of haemostasis after injury. Haemostasis has three major steps: a) vasoconstriction, b) temporary blockage of a break by a platelet plug, and c) blood coagulation, or formation of a fibrin clot. These processes seal the hole until tissues are repaired (Wikipedia 2015). So, the possible mechanism of action of the plant extracts might be due to the effects created on these steps.

In many previous studies, the aqueous,

Table1. Haemostatic efficacy of fresh plant extracts in wound of rabbit (n = 8).

Sl. No.	Plant name	Bleeding time after addition of fresh extract (seconds) (Mean \pm SE)
1.	<i>Artemisia nilagirica</i> (Clarke).	35.00 \pm 1.50
2.	<i>Barleria lupulina</i> Lindl.	30.00 \pm 2.34
3.	<i>Blumea lacera</i> Dc.	38.00 \pm 1.87
4.	<i>Croton bonplandianum</i> Baill. (leaf extract)	40.00 \pm 2.69
5.	<i>Croton bonplandianum</i> Baill. (latex)	10.00 \pm 1.22
6.	<i>Glinus oppositifolius</i> (L.) Aug. DC.	35.00 \pm 2.29
7.	<i>Mikania scandens</i> (L) Willd.	25.00 \pm 1.87
8.	Control (without any medicine/test medicine)	120.00 \pm 2.91
9.	Tr. Ferri. Per Chlor.(standard drug)	13.00 \pm 2.54

alcoholic and petroleum ether (Mehta *et al.*, 2013), hydro alcoholic extracts (Dandjesso *et al.*, 2012) of some dry plant parts were tested *in vitro* to validate the reported haemostatic effect of some other plant parts. *In vivo* validation report of plant extracts as haemostatic agent on rabbit wound is scarce.

CONCLUSION

The live plant leave extracts of *Artemisia nilagirica* (Clarke), *Barleria lupulina* Lindl., *Blumea lacera* Dc., *Croton bonplandianum* Baill, *Glinus lotoides* Lin. and *Mikania scandens* (L) Willd. can induce haemostasis in fresh wounds of rabbits. The latex collected from the wounded small branches of living plant of *Croton bonplandianum* Baill. is having highest activity of causing haemostasis than all the leaf extracts and control.

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